E. 5 Expression of final construct

22.08.2014

Preparing of different media for induction

Different 2YT media with different additives are prepared, to coinduce the final construct and the different chaperones. Additionally, media to express only the final construct or the chaperones are prepared as control.

Compound of the media:

Medium	**1**			**2**					
∑	250	mL		∑	250	mL			
CM 1:1000	250	µL		CM 1:1000	250	µL			
Amp 1:1000	250	µL		Amp 1:1000	250	µL			
Ara 4mg/mL	1000	mg		Ara 4mg/mL	1000	mg			
IPTG 100 µM	25	µL		Tet 100ng/ml	2500	µL			
				IPTG 100 µM	25	µL			
3				**4**			**5**		
∑	100	mL		∑	100	mL	∑	100	mL
CM 1:1000	100	µL		CM 1:1000	100	µL	CM 1:1000	100	µL
Amp 1:1000	100	µL		Amp 1:1000	100	µL	Amp 1:1000	100	µL
Ara 4mg/mL	400	mg		Ara 4mg/mL	400	mg	IPTG 100 µM	10	µL
Tet 100ng/ml	1000	µL							

Cotransformation of the final construct (PL 215-217) into competent cells already transformed with different chaperones. The final construct is transformed into the cells BL21 (C1-C5) and JM (C1-C5) two times for every cell type. One time into fresh competent cells and one time into competent cells that were already used and therefore defrosted, but that are also already characterized.

-Plating and over night culture on Ampicillin + Chloramphenicol plates.

23.08.2014

On all plates colonies have grown. The plates are stored at 4 °C.

All contransformed cell types with all chaperone constructs/Lac-end are inoculated for over night culture. The chaperone constructs are already induced in that culture. As control two cultures that contain only chaperones and one culture that contains only Lac-end are inoculated as well.

Chaperone	Induction	Per 5 mL
C1+Lac-end	L-Arabinose	20 mg
C2+Lac-end	L-Arabinose	20 mg
C3+Lac-end	L-Arabinose +Tetracycline	20 mg+25 µL (1:1000)
C4+Lac-end	L-Arabinose	20 mg
C5+Lac-end	Tetracycline	25 µL (1:1000)
C3 (control)	L-Arabinose +Tetracycline	20 mg+25 µL (1:1000)
Lac-end (control)	/	/

25.08.2014

Inoculation of liquid cultures of all 10 chaperon-strain combinations + lac-End construct in order to check for the production of the final construct with help of the chaperon kit.

26.08.2014

Inoculation of main culture and induction of lac-end at OD 0,5

Samples were taken after 0h, 3h, 6h and 24h.

02.09.2014

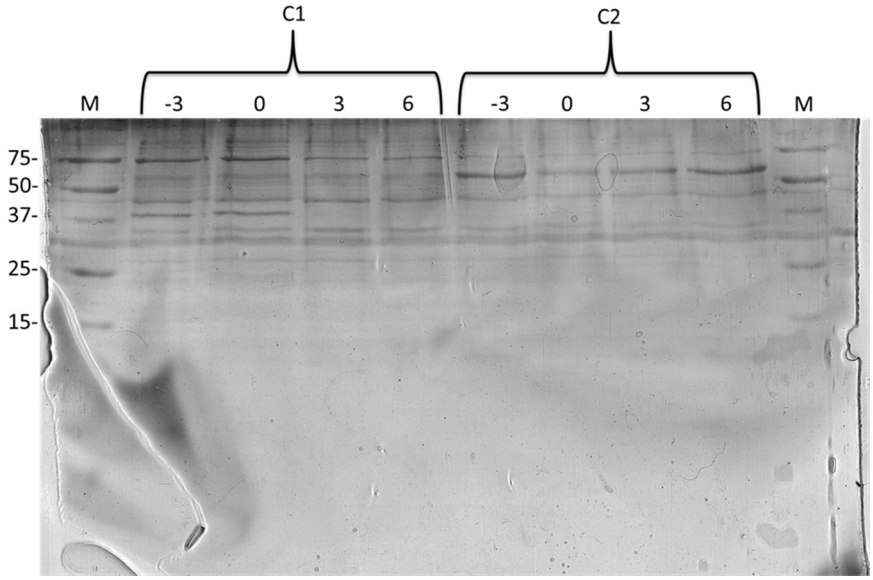

figure 1 SDS-Page of C1 and C2 0,3,6 hours after induction or rather 3 hours before induction with IPTG(-3). Ladder (M):Precision Plus Protein™ All Blue

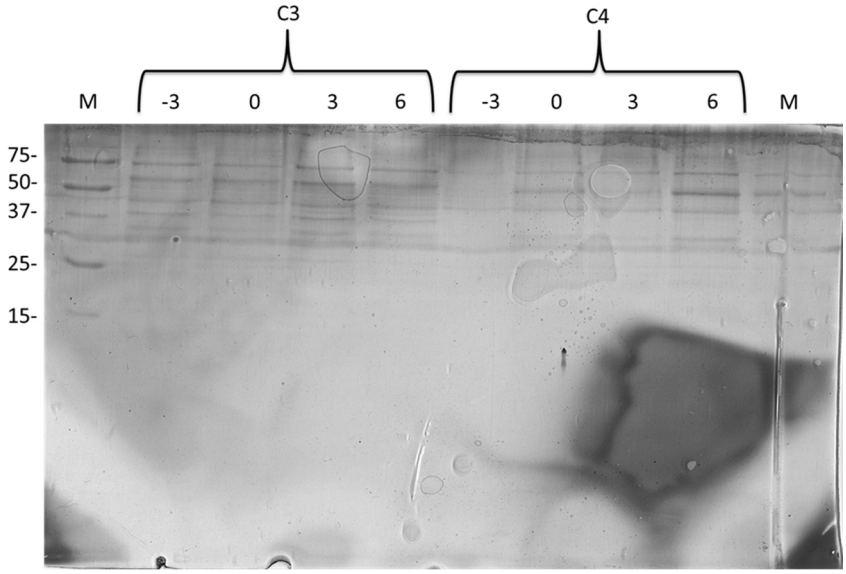

figure 2 SDS-Page of C3 and C4 0,3,6 hours after induction or rather 3 hours before induction with IPTG(-3). Ladder (M):Precision Plus Protein™ All Blue

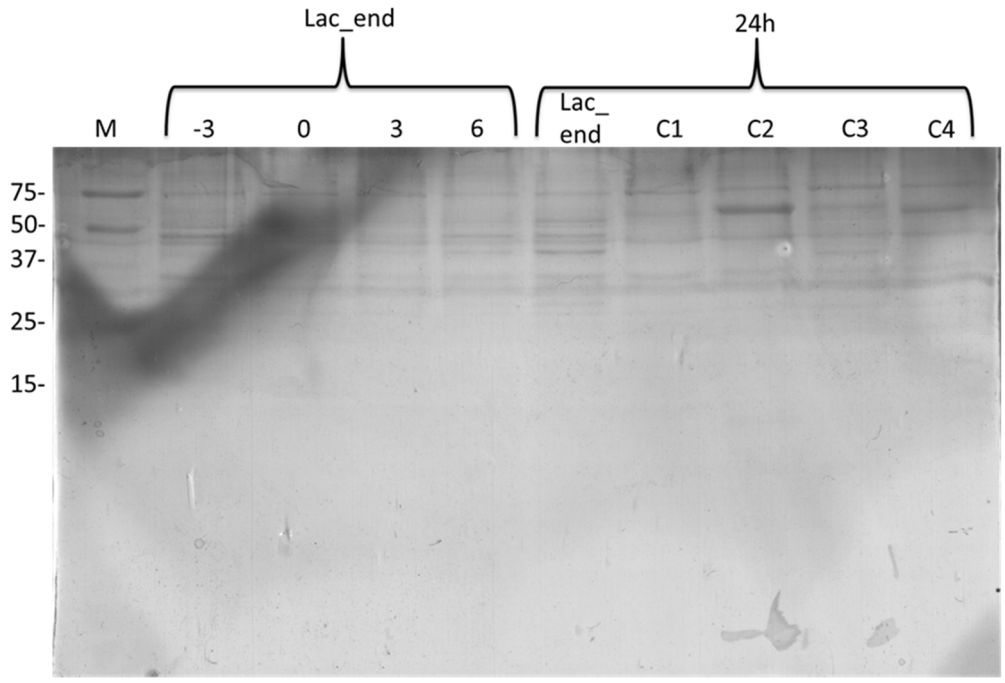

figure 3 SDS-Page of lac-end construct 0,3,6 hours after induction or rather 3 hours before induction with IPTG(-3) and lac-end construct, C1, C2 and C3 24h after induction. Ladder (M):Precision Plus Protein™ All Blue

→SDS-Pages show that chaperone expression decreases within a couple of hours. Therefore L-Arabinose is fed every 2 hours in the following experiments.